# METHOD OF RESOLUTION AND ANTIVIRAL ACTIVITY OF 1,3-OXATHIOLANE NUCLESOSIDE ENANTIOMERS

This application is a continuation of application U.S. Ser. No. 08/092,248, filed on Jul. 15, 1993, now abandoned, which is a continuation of U.S. Ser. No. 07/736,089, filed on Jul. 26, 1991, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/659,760, filed on Feb. 22, 1991, now U.S. Pat. No. 5,210,085, which is a continuation-in-part of U.S. Ser. No. 07/473,318, filed on Feb. 1, 1990, now U.S. Pat. No. 5,204,466.

U.S. Government has rights in this invention arising out of the partial funding of work leading to this invention through the National Institutes of Health Grant Nos. NIH 5-21935 and NIH AI-26055, as well as a Veteran's Administration Merit Review Award.

## BACKGROUND OF THE INVENTION

This invention is in the area of biologically active nucleosides, and specifically includes a method for the resolution of nucleoside enantiomers, including 1,3-oxathiolane nucleosides, and antiviral compositions that include the enantiomerically enriched 1,3-oxathiolane nucleosides, (−) and (+)-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC").

This application is a continuation-in-part application of U.S. Ser. No. 07/659,760, entitled "Method for the Synthesis, Compositions and Use of 2'-Deoxy-5-Fluoro-3'-Thiacytidine and Related Compounds", filed on Feb. 22, 1991, by Dennis C. Liotta, Raymond Schinazi, and Woo-Baeg Choi, that is a continuation-in-part application of U.S. Ser. No. 07/473,318, entitled "Method and Compositions for the Synthesis of BCH-189 and Related Compounds," filed on Feb. 1, 1990, by Dennis C. Liotta and Woo-Baeg Choi.

In 1981, acquired immune deficiency syndrome (AIDS) was identified as a disease that severely compromises the human immune system, and that almost without exception leads to death. In 1983, the etiological cause of AIDS was determined to be the human immunodeficiency virus (HIV). In December, 1990, the World Health Organization estimated that between 8 and 10 million people worldwide were infected with HIV, and of that number, between 1,000,000 and 1,400,000 were in the U.S.

In 1985, it was reported that the synthetic nucleoside 3'-azido-3'-deoxythymidine (AZT) inhibits the replication of human immunodeficiency virus type 1. Since then, a number of other synthetic nucleosides, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC), 3'-fluoro-3'-deoxythymidine (FLT), 2',3'-dideoxy-2',3'-didehydrothymidine (D4T), and 3'-azido-2',3'-dideoxyuridine (AZDU), have been proven to be effective against HIV. A number of other 2',3'-dideoxynucleosides have been demonstrated to inhibit the growth of a variety of other viruses in vitro. It appears that, after cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides are incorporated into a growing strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

In its triphosphate form, 3'-azido-3'-deoxythymidine is a potent inhibitor of HIV reverse transcriptase and has been approved by the FDA for the treatment of AIDS. However, the benefits of AZT must be weighed against the severe adverse reactions of bone marrow suppression, nausea, myalgia, insomnia, severe headaches, anemia, peripheral neuropathy, and seizures. These adverse side effects often occur immediately after treatment begins, whereas a minimum of six weeks of therapy is necessary to realize AZT's benefits. DDI, which has recently been approved by an FDA Committee for the treatment of AIDS, is also associated with a number of side effects, including sporadic pancreatis and peripheral neuropathy.

Both DDC and D4T are potent inhibitors of HIV replication with activities comparable (D4T) or superior (DDC) to AZT. However, both DDC and D4T are not efficiently converted to the corresponding 5'-triphosphates in vivo. Both compounds are also toxic and can cause peripheral neuropathies in humans.

The success of various 2',3'-dideoxynucleosides in inhibiting the replication of HIV in vivo or in vitro has led a number of researchers to design and test nucleosides that substitute a heteroatom for the carbon atom at the 3'-position of the nucleoside. Norbeck, et al., disclose that (±)-1-[(2β, 4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to below as (±)-dioxolane-T) exhibits a modest activity against HIV ($EC_{50}$ of 20 μm in ATH8 cells), and is not toxic to uninfected control cells at a concentration of 200 μm. *Tetrahedron Letters* 30 (46), 6246, (1989).

European Patent Application Publication No. 0 382 526 filed by IAF Biochem International, Inc. discloses a number of substituted 1,3-oxathiolanes with antiviral activity, and specifically reports that the racemic mixture (about the C4'-position) of the C1'-β isomer of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (referred to below as (±)-BCH-189) has approximately the same activity against HIV as AZT, and no cellular toxicity at therapeutic levels. (±)-BCH-189 has also been found to inhibit the replication of AZT-resistant, HIV isolates from patients who have been treated with AZT for longer than 36 weeks.

To market a nucleoside for pharmaceutical purposes, it must not only be efficacious with low toxicity, it must also be cost effective to manufacture. An extensive amount of research and development has been directed toward new, low cost processes for large scale nucleoside production. 2',3'-Dideoxynucleosides are currently prepared by either of two routes: derivatization of an intact nucleoside or condensation of a derivatized sugar moiety with a heterocyclic base. Although there are numerous disadvantages associated with obtaining new nucleoside analogues by modifying intact nucleosides, a major advantage of this approach is that the appropriate absolute stereochemistry has already been set by nature. However, this approach cannot be used in the production of nucleosides that contain either nonnaturally occurring bases or nonnaturally occurring carbohydrate moieties (and which therefore are not prepared from intact nucleosides), such as 1,3-oxathiolane nucleosides and 1,3-dioxolane nucleosides.

When condensing a carbohydrate or carbohydrate-like moiety with a heterocyclic base to form a synthetic nucleoside, a nucleoside is produced that has two chiral centers (at the C1' and C4'-positions), and thus exists as a diasteromeric pair. Each diastereomer exists as a set of enantiomers. Therefore, the product is a mixture of four enantiomers.

It is often found that nucleosides with nonnaturally-occurring stereochemistry in either the C1' or the C4'-positions are less active than the same nucleoside with the stereochemistry as set by nature. For example, Carter, et al., have reported that the concentration of the (−)-enantiomer of carbovir (2',3'-didehydro-2',3'-dideoxyguanosine) required to reduce the reverse transcriptase activity by 50% ($EC_{50}$ )